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Search History

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L2	L1 and "culturing cells"	3	L2
L1	"polycationic compounds" and "mammalian cells"	47	L1

END OF SEARCH HISTORY

L2: Entry 3 of 3 File: USPT May 6, 1997

DOCUMENT-IDENTIFIER: US 5627159 A
TITLE: Enhancement of lipid cationic transfections in the presence of serum

Abstract Text (1):
A method is provided for enhancing transfection efficiency of eukaryotic cells comprising contacting said cells with a lipid aggregate comprising nucleic acid and a polycationic lipid composition in the presence of a polycationic compound and serum. The polycationic compound is preferably Polybrene.TM., and the lipid aggregate preferably comprises liposomes of nucleic acid and LipofectAMINE.TM..

Brief Summary Text (5):
U.S. Pat. No. 5,286,634 of Stadler et al. for "Synergistic Method for Host Cell Transformation" issued Feb. 15, 1994 discloses the use of a polycationic compound to treat a host cell for a period of time prior to treating with a DNA-liposome complex to improve transformation of the cell. The invention was exemplified using plant cells which do not require serum in culture media or in vivo. The method of said patent is not believed effective in enhancing transfection of mammalian cells because the preferred polycationic compound of said patent (Polybrene.TM.), is toxic to mammalian cells in the absence of serum.

Brief Summary Text (6):
A problem with transfection of eukaryotic cells by means of liposomes is the fact that culturing such cells in vitro requires the use of serum in the medium for best results, and the use of serum in culture media is standard in the art. However, the use of serum in the culture medium substantially inhibits the efficiency of liposome transfection. Further, in the transfection of animal cells in vivo, serum is inherently present, again with an inhibiting effect on the efficiency of liposome transfection. Therefore a need exists for a method of eukaryotic transfection in the presence of serum which counteracts the inhibiting effects of the serum.

Detailed Description Text (2):
A method is provided for enhancing transfection efficiency of eukaryotic cells comprising contacting said cells with a lipid aggregate comprising nucleic acid and a cationic lipid in the presence of a polycationic compound and serum. The polycationic compound is preferably Polybrene.TM..

Detailed Description Text (3):
The polycationic compound is present at a concentration of about 20 to about 80 .mu.g/ml, more preferably between about 20 and about 40 .mu.g/ml. The lipid aggregate, which is composed of liposomes of nucleic acid and cationic lipid, preferably comprises between about 5 and about 20 .mu.l per ml, more preferably between about 12 and about 15 .mu.l per ml.

Detailed Description Text (6):
In the preferred embodiment of this invention, the polycationic compound is Polybrene.TM. and the lipid aggregate comprises cationic liposomes of LipofectAMINE.TM. and DNA.

Detailed Description Text (12):
"Polycationic compound" include hexadimethrine bromide (Polybrene.TM.) or other salts of hexadimethrine as a preferred species. This term also includes compounds such as the salts of poly-L-ornithine, poly-L-arginine, poly-L-lysine, poly-D-lysine, polyalylamine and polyethyleneimine. In addition, any polycationic compound prepared from the combination of any compound containing at least two good leaving groups, such as dihalogenated compounds especially dibromide and diiodide, but less desirably difluoride, or ditosylates and any poly(tetraalkyl-substituted amine) containing chains of two or more carbons between the amine groups could also serve as an effective polycationic compound of this invention. In the instance of the salt of hexadimethrine, the compound containing two good leaving groups could be 1,3 dibromopropane and the poly(tetraalky) substituted amine is N,N,N',N'-tetramethylhexamethylene diamine. In this case the polyamine is a diamine. Indeed, almost any combination of alkyl or xylyl groups attached to a basic carbon chain and combined using the aforementioned good leaving groups would be effective polycations. However, for reasons regarding weakening the basicity of the compound, it is believed that aryl groups attached directly to nitrogen compounds would be less effective than the foregoing compounds.

Detailed Description Text (14):
Eukaryotic cells, as known to the art, are cells with visibly evident nuclei, such as plant and animal cells, more preferably animal cells including insect cells, and mammalian cells, more preferably human cells and higher mammalian cells.

Detailed Description Text (15):
Serum, as is known to the art, is the watery portion of an animal fluid remaining after coagulation. Preferably the serum used herein is mammalian blood serum, more preferably sera commercially available for use in the culturing of mammalian cells, and most preferably is fetal bovine serum (FBS).

Detailed Description Text (16):
The cells being transfected may be contacted with the polycationic compound of this invention by first treating the cells with said compound to form a complex with said cells prior to contacting with the lipid aggregate comprising nucleic acid, or by mixing the polycationic compound with the lipid aggregate comprising nucleic acid prior to contacting the cells with this mixture.

Detailed Description Text (20):
A panel of polycationic compounds was screened in LipofectAMINE.TM. transfections in the presence of serum, and Polybrene.TM. was selected as a preferred polycationic compound of this invention since it showed higher transfection frequency and lower cell toxicity (data not shown). Additionally, Polybrene.TM. is an FDA-approved reagent for gene therapy.

Detailed Description Text (21):
The continued presence of serum during transfections has advantages in several circumstances. Loss of cells during the transfection is diminished, and stimulation of gene expression by the altered levels of serum hormones can be avoided. Previous reports of serum inhibition of cationic lipid-mediated transfections (Felgner, P. L. and Ringold, G. M. (1989) Nature 337, 387) have suggested that the inhibition was due to the presence of sulphated proteoglycans in the serum. Transfections with monocationic lipid reagents are not inhibited by serum when DNA-lipid complexes are made in serum-free conditions (Brunette, E., et al. (1992) Nuc. Acids Res. 20:1151; Ciccarone, V., et al. (1993) Focus 15, 80). The inhibition of LipofectAMINE.TM. transfections by serum occurs even when complexes are made in serum-free conditions (Ciccarone, V., et al. (1993) Focus 15, 80; Hawley-Nelson, P., et al. (1993) Focus 15:73). This inhibition now can be overcome by addition of optimal concentration polycationic compounds such as the polycationic reagent Polybrene.TM..

CLAIMS:

1. In a method of transfecting an animal cell in the presence of serum, comprising contacting said cell with a lipid aggregate comprising nucleic acid and a cationic lipid, wherein the improvement comprises: contacting said cell with said lipid aggregate in the presence of a polycationic compound, thereby transfecting said animal cell with said nucleic acid.

2. In a method of transfecting an animal cell in the presence of serum, comprising contacting said cell with a lipid aggregate comprising nucleic acid and a cationic lipid, wherein the improvement comprises: either (a) first contacting said cell with a polycationic compound to form a complex of said cell with said polycationic compound followed by contacting said cell complex with said lipid aggregate; or (b) first contacting said lipid aggregate with said polycation compound to form a mixture followed by contacting said cell with said mixture, thereby transfecting said animal cell with said nucleic acid.

3. A method of claim 2 wherein said polycationic compound is POLYBRENE.TM..

5. A method of claim 2 wherein said cells are mammalian cells.

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